Antiviral Research, 1 (1981) 367–381
Elsevier Biomedical Press

HUMAN β-TYPE INTERFERON ENHANCES THE EXPRESSION AND SHEDDING OF Ia-LIKE ANTIGENS. COMPARISON TO HLA-A,B,C AND β_2-MICROGLOBULIN

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(Received 29 July 1981; accepted 24 August 1981)

Expression and shedding of Ia-like antigens were evaluated in human cell lines treated with β-type interferon. A significant and dose-dependent increase in the amount of cell-associated antigens was found that reached a peak after 48 h of interferon treatment. An increased accumulation of Ia antigens in the culture fluids was also observed. Similar increases in the expression and shedding of HLA-A,B,C and β_2-microglobulin were observed. Human α-type interferon caused enhanced expression as well as enhanced shedding of HLA-A,B,C and β_2-microglobulin, but was ineffective with respect to Ia expression.

interferon Ia antigens HLA-A,B,C β_2-microglobulin

INTRODUCTION

In addition to their well-known antiviral effects, interferons (IFN) have been found to exert a variety of biological activities. These include inhibition of cell division [27, 39], modulation of erythroid differentiation [7, 35, 36], enhancement of phagocytosis [16], altered expression of cell surface antigens [12], and modulation of the immune system. Among the latter, inhibition of antibody responses in culture [17], enhancement of cytotoxicity of sensitized lymphocytes [24], augmentation of cytotoxic activity of natural killer cells [14, 37, 48], and the ability to suppress cell-mediated responses in vivo [6] and in vitro [18], have been described. Furthermore, it is known that IFN acts on cell membrane components [25], including products of the major histocompatibility complex (MHC). Studies on the H-2 system [26, 38, 49] were carried out, both in vivo and in vitro, on thymocytes, lymphoid cells and embryo fibroblasts. More recently, similar studies were performed in human lymphoid cells, using cytotoxicity assays, cell sorter immunofluorescence analysis and autoradiography [10, 15]. IFN doses used in those studies ranged from 10^2 to 10^4 units/ml.

We have studied the influence of human β-type IFN (HuIFN-β) on the expression as well as on the shedding of Ia and other MHC products in a wide spectrum of human cell lines.

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Ia-like (class II products) and HLA-A,B,C (class I products) as well as β_2-microglobulin (β_2-m) were quantitatively determined. β_2-m, though not being under the genetic control of the HLA region, nevertheless is associated to both HLA-A,B,C chains and to TL-A chains [44] and may possess regulatory functions on their expression on the cell surface. All determinations were performed by radioimmunoassay (RIA) based on inhibition of the binding reaction between purified ^{125}I-labelled antigens and the corresponding specific antibodies [45, 46]. By using this sensitive and reproducible method, which allows the determination of both cell-associated and cell-shed products, we were able to demonstrate that HuIFN-β can influence the expression and the shedding of Ia antigens.

MATERIALS AND METHODS

Cells

The following cell lines and strains were used: E_1 SM and ME, human diploid cell strains derived from embryonic skin and muscle, and selected for production of interferon [4, 20]; MG-63, a human fibroblastoid cell line derived from an osteosarcoma [4]; Namalva and Molt-4, both lymphoblastoid cell lines [22, 30] of B- and T-cell origin respectively; HeLa and HEp-2, epithelioid cell lines derived from a cervical carcinoma and a larynx carcinoma, respectively [51]; M10 and M14, both cell lines derived from human melanoma [28].

Interferons

Crude human fibroblast IFN was prepared on E_1 SM or ME cells, by induction with Newcastle disease virus [7], or by the superinduction procedure with polyriboinosinic-polyribocytidylic acid, cycloheximide and actinomycin D [5]. IFN titers ranged from 10^4 to 10^5 units/ml, with a specific activity of $10^5-10^{5.2}$ international units (I.U.)/mg protein. This IFN was partially purified by ammonium sulphate precipitation as previously described [47]. The preparation so obtained had a specific activity of 10^6 I.U./mg protein. We also used samples of partially purified fibroblast IFN kindly provided by Dr. M. De Ley, Rega Institute, Leuven, Belgium, with a specific activity of 10^6 I.U./mg protein. HuIFN-β purified to homogeneity (preparation 34370) and anti-HuIFN-β goat antiserum (preparation 925) were a generous gift from Professor Dr. A. Billiau, Rega Institute, Leuven, Belgium. The serum was raised against pure HuIFN-β; its titer was 10^{-7} against 10 I.U./ml of HuIFN-β.

Partially purified leukocyte IFN was obtained from Dr. K. Cantell, Central Public Health Laboratory, Helsinki, through the courtesy of Dr. G.B. Rossi, Istituto Superiore di Sanità, Rome, Italy. It had a specific activity of 10^6 I.U./mg protein.

IFN was titrated by a CPE inhibition method on E_1 SM cells, grown in microtiter plates, using vesicular stomatitis virus (VSV) as a challenge. Throughout this paper 1 unit/ml of IFN is defined as the concentration causing 50% CPE inhibition in our assay system.

In this assay system, 1 unit of fibroblast IFN corresponded to 2 I.U.; 1 unit of leukocyte IFN corresponded to 0.5 I.U. In a typical experiment, cells were seeded at half the saturation density, and IFN was given at the time of cell seeding. Cells were collected after 24–48 h, washed twice with phosphate-buffered saline (PBS) and stored at -20°C. Parallel cultures were trypsinized and counted.

Determination of the antiviral state

24 h after addition of IFN, the cells were inoculated with VSV (5 plaque-forming units (p.f.u.)/cell, as assessed by titration on human HEp-2 cells). After 1 h adsorption at 37°C, the inoculum was discarded and replaced by fresh medium. VSV was collected 18 h post-infection, and the yield was titrated by plaque assay on mouse L_{929} cells.

^{125}I-labelled antigens

Purified β_2-m was a gift from Dr. N. Tanigaki (Rosswell Park Memorial Institute, Buffalo, NY). It showed a single 12,000 dalton band on electrophoretic analysis.

Papain-solubilized HLA-A,B,C antigens were prepared from the lymphoid cell line RPMI 1788, by the isolation procedure described earlier [31]. On electrophoretic analysis, it showed only two bands of 34,000 and 12,000 daltons, respectively. The heavy and light polypeptides were separated by acid dissociation of this material [2]. Chemical purity was confirmed by the presence of only one N-terminal and one C-terminal amino acid residue of the heavy chain [13].

Detergent-purified human Ia molecules were obtained from Renex-30-solubilized membranes of U698M cells, as previously described [45, 46]. Ia preparations, devoid of HLA-A,B,C and β_2-m molecules [43], on electrophoretic analysis showed the two-band pattern (27,000 and 34,000 daltons respectively) typical of Ia molecules.

The antigens were labelled according to methods described earlier [31, 46].

Inhibitors

Either cell lysates or culture fluids were used as inhibitors in RIA. Cells were washed twice with PBS, and resuspended in 0.075 M Tris-HCl, pH 7.8, containing 2% Renex-30. Culture fluids were tested after extensive dialysis against 0.075 M Tris-HCl, pH 7.8, and addition of Renex-30 (2% final concentration).

Antisera

Rabbit anti-human Ia serum, preparation 7147-2, was used. It was raised against glycoproteins of Daudi cells. Its specificity, as previously described [41], is directed against both α and β Ia subunits. Rabbit anti-human HLA-A,B,C serum, preparation 6095, was raised against purified antigens of RPMI 1788 cells, as described [33]. Both anti-Ia and anti-HLA-A,B,C sera were kindly provided by Dr. N. Tanigaki.

Rabbit anti-human β_2-m serum, preparation 5895, was raised against a purified preparation of human β_2-m as described earlier [32].

Radioimmunoassays

A direct binding test was used [45], as previously done for the quantitative determinations of β_2-m and HLA-A,B,C antigens in subcellular fractions of cultured human cells [34] and for the determination of the organ and tissue distribution of human Ia-like antigens [23]. Antiserum dilutions were chosen so as to give about 60% of the maximum binding in the absence of the inhibitor. The dilutions were made in 0.075 M Tris-HCl, pH 7.8, containing 0.5% Renex-30 and 0.02% bovine serum albumin (diluent 1). Aliquots of 20 μl of diluted antiserum were used in each test. Inhibitors were added and the volumes adjusted to 100 μl with diluent 1, containing 2% Renex-30. After incubation for 1 h at 37°C of antiserum and inhibitors, the labelled reference antigen (15,000 c.p.m. in 20 μl of diluent 1) was added. After a further incubation for 1 h at 37°C, sheep anti-rabbit Ig serum at equivalence was added. To ensure the presence of a visible precipitate, normal serum was added to reach a total serum volume of at least 4 μl. Controls without inhibitor and with normal serum instead of antiserum were included. After 40 min at 37°C, 2 ml of borate-buffered saline (sodium borate, 0.02 M, pH 7.8) was added to each tube. After centrifugation at room temperature for 10 min at 400 × g, most of the supernatant was removed by suction; the sediment was resuspended, and an additional 2 ml aliquot of washing solution was added. After a second centrifugation and removal of most of the supernatant, the tubes were counted for radioactivity by a gammacounter. Inhibition was expressed by the following formula:

$$\%\text{inhibition} = 100 - \frac{\text{c.p.m. bound with inhibitor} - \text{c.p.m. bound by normal rabbit serum}}{\text{c.p.m. bound without inhibitor} - \text{c.p.m. bound by normal rabbit serum}} \times 100$$

Each inhibitor was tested in different dilutions and the amount corresponding to 50% inhibition was estimated. This amount was taken as 1 I.U.$_{50}$. It must be pointed out that the I.U.$_{50}$ of the three products tested are arbitrary units, and are therefore not comparable with each other. For β_2-m 1 I.U.$_{50}$ corresponded to 0.6 ng.

It was previously shown that no inhibition by extracts of Daudi cells (which are devoid of HLA-A,B,C antigens) was observed on the anti-HLA-A,B,C serum activity (see Table 3 of ref. 23).

A lysate from M14 cells (expressing all the three antigens) was divided in aliquots and added as a standard sample in each RIA. Values obtained in four different experiments were sufficiently reproducible, as the variation coefficient (standard deviation/mean) never exceeded 15% for each of the three antigens tested. Accordingly, the experimental values obtained in different experiments were not corrected for fluctuation of assay sensitivity.

RESULTS

Influence of β-type IFN on growth, viability and antiviral state of cultures

A panel of cell lines (Table 1) was chosen to represent a variety of cell types: epithelioid cells (M10, M14, HEp-2 and HeLa); B- and T-lymphoblastoid lines (Namalva and Molt-4 respectively); and fibroblastoid cells (E_1 SM and MG-63).

As the present report was also aimed at studying the effects of IFN on the release of membrane antigens in culture fluids, it was crucial to assess the viability of the cell cultures exposed to IFN treatment. In fact, accumulation of membrane antigens in the fluids can be brought about either by their release from the plasma membrane of a viable cell (i.e. 'shedding'), or as a consequence of cell death.

As shown in Table 1, treatment with 5000 units/ml of partially purified HuIFN-β did not cause a decrease in cell counts as compared to the seeding cell concentrations. Viability, as assessed by nigrosin exclusion, was not significantly affected either, as it ranged between 90% and 97%, regardless of IFN treatment. Moreover, HuIFN-β, within the dose range used, did not affect overall protein synthesis (data not shown). The only exception was the T-lymphoid cell line, Molt-4, whose viability was significantly affected (79.1%) by as little as 1500 units/ml of IFN. That crude type I IFN induces a strong reduction of viability of mouse T-lymphoid cells has already been reported [38]. An antiviral state, measured by reduction of VSV yields, was effectively induced by IFN in all cell lines tested, and closely paralleled the effects of IFN on the expression of histocompatibility antigens. Treatment with 5000 units/ml of HuIFN-β resulted in a significant inhibition of VSV replication, ranging from 1.9 to 5.4 $\log_{10}$, as compared to VSV yields in control cultures (Table 1).

Cellular expression and shedding of Ia molecules, HLA-A,B,C, and β_2-m in different cell lines

Cell lysates and dialyzed supernatants from the cell lines listed in Table 1 were tested by RIA for β_2-m, HLA-A,B,C and Ia. The results are shown in Table 2. All cell lines expressed and most of them released β_2-m and HLA-A,B,C. Ia antigens were expressed only by Namalva, M10 and M14 cell lines. The cellular concentration of each antigen appeared to vary over a 10–20-fold range among the different cell lines. The variability between cell lines was even more apparent in the assays on supernatant fluids. As a general rule, the extent of shedding of Ia and HLA-A,B,C was low, and that of β_2-m high when compared to the corresponding expression at the cell level. HEp-2 cells exhibited an exceptionally high HLA-A,B,C shedding. M10 cells shed comparatively low amounts of β_2-m: in this cell line, shedding of Ia was also low, in spite of its high expression at the cell level. In the evaluation of these results, moreover, it must be kept in mind that values were expressed as inhibition units in RIA and thus are comparable only within each antigenic system.

TABLE 1

Effects of fibroblast IFN on viability, growth and response to viral infection of different cell lines

Cell line or strain			% Viability		Cell growth[a]		VSV yield (log_{10} p.f.u/ml)	
Designation	Morphology	Origin	Control	IFN-treated	Control	IFN-treated	Control	IFN-treated
M14	Epithelioid	Melanoma	97.2	93.5	1.1	1.0	7.5	4.7
M10	Epithelioid	Melanoma	94.8	96.1	1.0	1.0	8.4	6.2
HEp-2	Epithelioid	Larynx carcinoma	96.2	94.5	1.2	1.0	8.6	5.9
HeLa[b]	Epithelioid	Cervical carcinoma	97.1	96.3	1.1	1.0	8.9	7.0
Namalva	B lymphoid	Burkitt lymphoma	97.4	94.2	1.7	1.7	6.8	4.5
Molt-4[c]	T lymphoid	Thymoma	90.0	79.1	1.0	0.8	7.7	4.8
E_1 SM	Fibroblast	Normal embryonic skin and muscle	96.2	94.1	1.0	1.0	10.3	4.9
MG-63	Fibroblast	Osteosarcoma	95.1	96.3	1.3	1.1	9.3	5.8

Cells were grown in the presence of 0 or 5000 units/ml of partially purified fibroblast IFN for 24 h.

[a] Ratio of viable cell count at 24 h over cell count at time zero.

[b] 5000 units/ml of crude fibroblast IFN.

[c] 1500 units/ml of partially purified fibroblast IFN.

TABLE 2

Effect of treatment with fibroblast IFN on expression and shedding of Ia molecules, HLA-A, B, C antigens and β_2-m in different cell lines or strains

Cell line or strain	Fluid tested	Levels ($I.U._{50}/10^6$ cells) of								
		Ia-like molecules			HLA-A,B,C antigens			β_2-m		
		Control	IFN-treated[a]	Ratio	Control	IFN-treated[a]	Ratio	Control	IFN-treated[a]	Ratio
M14	Cell lysate	39	58	1.5	12	22	1.8	85	200	2.3
	Culture fluid	10	13	1.3	10	15	1.5	115	268	2.3
M10	Cell lysate	402	480	1.2	14	25	1.8	84	158	1.9
	Culture fluid	12	18	1.5	10	19	1.9	44	88	2.0
HEp-2	Cell lysate	0[b]	0		78	113	1.4	133	191	1.4
	Culture fluid	0	0		1400	1850	1.3	269	492	1.8
HeLa[c]	Cell lysate	0	0		12	15	1.2	17	45	2.6
	Culture fluid	0	0		0	0		203	308	1.5
Namalva	Cell lysate	19	25	1.3	13	20	1.5	11	20	1.8
	Culture fluid	0	0		0	0		85	103	1.2
Molt-4[d]	Cell lysate	0	0		4	10	2.5	18	56	3.1
	Culture fluid	0	0		ND[e]	ND		ND	ND	
E_1 SM	Cell lysate	0	0		20	48	2.4	39	97	2.5
	Culture fluid	0	0		0	0		217	538	2.5
MG-63	Cell lysate	0	0		32	62	1.9	40	159	4.0
	Culture fluid	0	0		0	5.3		166	252	1.5

[a] Partially purified fibroblast IFN (5000 units/ml, 24 h).

[b] 0 designates levels below detection limit.

[c] Treated with 5000 units/ml of crude fibroblast IFN.

[d] Treated with 1500 units/ml of partially purified fibroblast IFN.

[e] Not determined. This line showed marked loss of viability in the presence of IFN.

Influence of fibroblast IFN on the cellular expression and on shedding of Ia, HLA-A,B,C and β_2-m

A preliminary screening of the effects of β-type IFN on the cell line panel was performed under fixed conditions (5000 units/ml, 24 h, 37°C). Table 2 shows that the ratios of values observed with treated vs. untreated cells were constantly above 1. Thus the IFN treatment caused increased expression of Ia, HLA-A,B,C and β_2-m. However, it did not induce the appearance of Ia antigens in Ia-negative cell lines. For each antigen the magnitude of enhancement varied with the cell line tested. The highest increases were found for either cell-bound or shed β_2-m expression, while Ia and HLA-A,B,C expression was enhanced at a lower extent.

It is unlikely that the enhancing effect on expression of histocompatibility antigens was due to contaminants present in the IFN preparations, since the partially purified IFN was more effective than the crude preparation, as also seen in other systems [36] (not shown).

The effect of IFN on MHC products was also observed when M14 cells were treated with pure HuIFN-β, and was completely abolished by preincubating pure IFN with the specific antiserum (Table 3). The same treatment completely abolished the antiviral activity.

Time-course experiments

Two lines, Namalva and M14, which express all three antigens studied, were selected for a more detailed study of the interferon effects. Cell cultures were given 5000 units/ml of fibroblast IFN at the time of cell seeding; cells and supernatants were collected at different time intervals, and tested for antigen expression. Data from these experiments were calculated both as $I.U._{50}/10^6$ cells and $I.U._{50}$/mg of cellular protein. Similar time-course patterns were obtained either way, thus indicating that the observed effects could not be attributed to an increase in cell size.

TABLE 3

Effect of treatment with pure HuIFN-β with/without anti-HuIFN-β serum on the expression of MHC products and on the response to virus infection in M14 cells

Treatment	Levels ($I.U._{50}/10^6$ cells) of			VSV yield ($\log_{10}$ p.f.u./ml)
	Ia molecules	HLA-A,B,C antigens	β_2-m	
None	38.1	18.7	77.2	7.9
HuIFN-β	79.8	79.7	292.4	3.2
HuIFN-β + antiserum	37.8	18.1	85.0	8.0

Cells were seeded in the presence of 4000 I.U./ml of pure HuIFN-β preincubated with/without a 10-fold excess of goat-specific antiserum at 4°C for 90 min. After 48 h cultures were processed as in Methods.

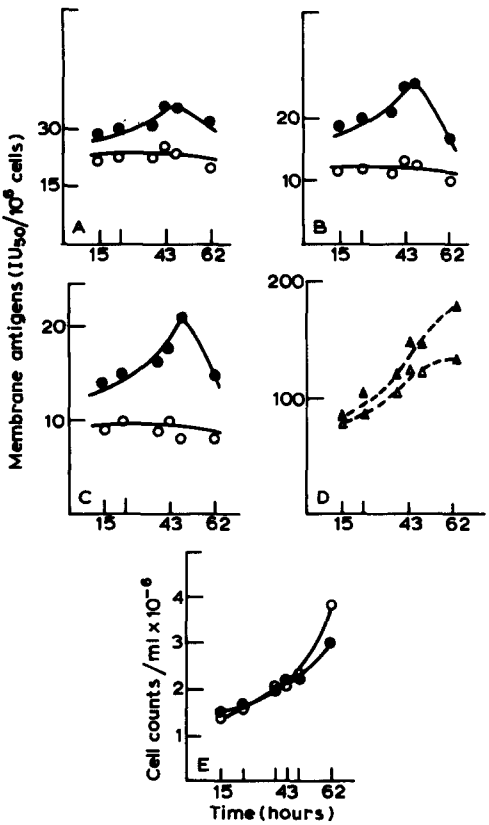

Fig. 1. Time course of the effect of fibroblast IFN on histocompatibility antigens in Namalva cells. A) Ia molecules in cell lysates. B) HLA-A,B,C antigens in cell lysates. C) β_2-m in cell lysates. D) β_2-m in culture fluids. E) Cell growth. ○, △, untreated; ●, ▲, 5000 units/ml of partially purified IFN starting at cell seeding.

Fig. 1 shows the data obtained with Namalva cells. Untreated cultures showed constant antigen concentrations on the cells and some accumulation of shed β_2-m products over 62 h. Cell-associated antigen expression was increased after IFN treatment, reaching a peak after 48 h of culture, with a subsequent decline. β_2-m accumulated in the fluids of IFN-treated cells to a higher extent than in those of untreated cells. As shown in Fig. 1, cell growth in the presence of IFN was unmodified until 48 h, decreasing only slightly thereafter, indicating that even in the presence of IFN cells underwent at least one cell doubling.

A similar pattern was observed with M14 cells (Fig. 2). It can be noted that, while cell-bound antigens in IFN-treated cultures showed a peak of increased expression and then tended to decline, a progressive accumulation of shed products took place, reaching levels considerably higher than those of controls. Quantitatively, IFN-induced increase of Ia and HLA-A,B,C was predominantly on cell-bound antigens, whereas that of β_2-m was predominantly on shed products. On the other hand, the increase was more evident

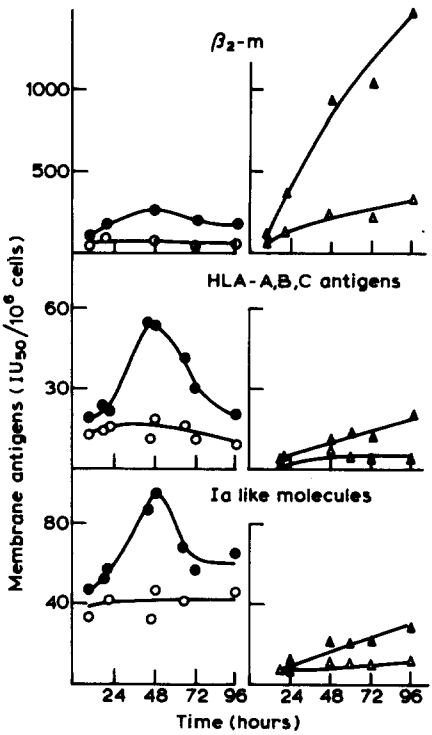

Fig. 2. Time course of the effect of fibroblast IFN on M14 cells. ○, △, untreated cultures; ●, ▲, cultures treated with 5000 units/ml of partially purified IFN starting at cell seeding. ○, ●, cell lysates; △, ▲, culture fluids. *P* values (Student's *t* test on quadruplicate determinations) for cell lysates (48 h) were: β_2-m, $P < 0.02$; HLA-A,B,C, $P < 0.02$; Ia, $P < 0.01$. For culture fluids (72 h): β_2-m, $P < 0.01$; HLA-A,B,C, $P < 0.01$; Ia, $P < 0.05$.

on β_2-m than on Ia and HLA-A,B,C (see Tables 2–3 and Figs. 1–3). Comparable results were obtained when fibroblast IFN was added one or more days after cell seeding. Thus, the lag phase was intrinsic to the IFN effect, and not related to the lag in the cell growth that usually follows trypsinization and/or seeding.

Dose dependence of IFN effects; comparison of β- and α-type IFN

M14 cells were incubated with varying concentrations, ranging from 500 to 5000 units/ml, of either fibroblast or leukocyte IFN for 48 h. Cell lysates and dialyzed supernatants were tested for Ia, HLA-A,B,C and β_2-m. As shown in Fig. 3, maximal enhancement was observed when the cells were treated with IFN concentrations ranging from 2000 to 5000 units/ml. No substantial difference was found between optimal IFN concentration for shedding and for cellular expression of the same antigen. A similar dose dependence was found in all cell lines tested.

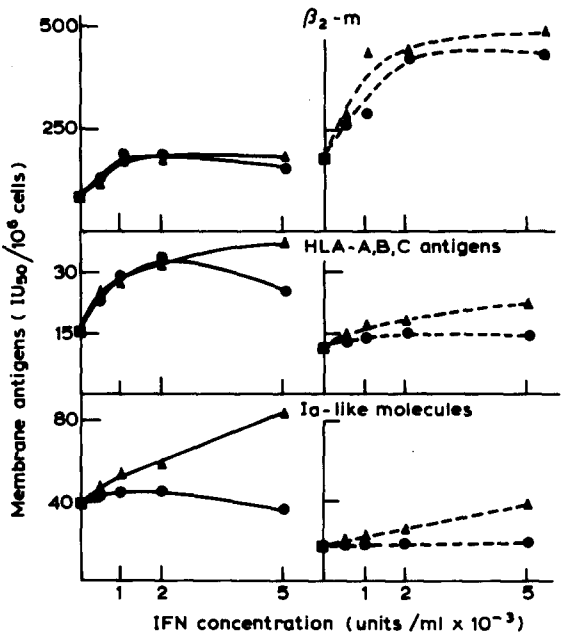

Fig. 3. Comparison of the effect of leukocyte and fibroblast IFN on cell membrane antigen expression in M14 cells: dose-response curves. Cultures were seeded in medium containing increasing doses of IFN and incubated at 37°C for 48 h. A) Ia molecules. B) HLA-A,B,C antigens. C) β_2-m. •, Leukocyte IFN; ▲, fibroblast IFN; ———, cell lysates; ------, culture fluids.

The increased expression of Ia antigens described above contrasted with previously reported data which suggested that IFN enhanced only β_2-m and HLA-A,B,C (cellular) expression, but not Ia expression [3, 10, 26]. The possibility was considered that the disagreement could be due to the use of different types of IFN. It is possible, indeed, that different types of IFN exert different activities on histocompatibility antigens.

In the experiment shown in Fig. 3, no major differences were observed in the effects of leukocyte and fibroblast IFN, as far as HLA-A,B,C and β_2-m were concerned, with respect to either cell-bound or shed products. Shedding and expression of Ia was increased by fibroblast but not by leukocyte IFN.

DISCUSSION

Enhanced expression of H-2 antigen and β_2-m has been described in IFN-treated murine lymphoid cells [25, 26, 38, 49]. Also, human leukocyte IFN has been shown to enhance expression of HLA-A,B,C antigens and β_2-m in human lymphoid cells [10, 15]. In the present study, we extend the latter observation to human fibroblast IFN, implying that not only HuIFN-α but also HuIFN-β has the potential to increase the expression of these cell membrane components. Furthermore, enhanced expression was not only seen in cells of lymphoid origin, i.e. cultured lymphoblastoid cells, but also in cell cultures of fibroblastoid and epithelioid origin.

Human fibroblast IFN was found to increase the expression and release of Ia antigens. In studies done by other authors [3, 10, 26], leukocyte IFN failed to affect Ia, a finding which was confirmed by us. It must be noted that IFN treatment enhanced Ia antigen expression only on cells which already expressed these antigens, indicating that IFN did not switch on inactive genes but only increased the expression of already activated genes. The kinetics of enhanced expression of Ia antigens by β-type IFN were similar to those of enhanced expression of HLA-A,B,C antigens and β_2-m. The conclusions from these results are that IFN-induced enhancement of expression and release of antigenic cell membrane components is not confined to cells of lymphoid origin, and, secondly, that different types of IFN may affect cell membrane components in different ways, and may therefore have specific effects on the interactions in vivo between cells involved in immune responses. It may be relevant in this respect to note that Ia antigens are likely to be products of immune response (Ir) genes [19]; moreover, expression of HLA-A,B,C antigens is involved in T-lymphocyte killing of virus-infected cells [52].

We have also determined the effects of IFN on shedding into the culture medium of the three membrane components under study. For the following reasons we have assumed that antigens present in the culture medium represent true secretion and not leakage due to cell death: 1) a high degree of cell viability was maintained throughout the experiments; 2) the concentration of antigens released into the cell culture fluid was up to 16-fold higher than could be accounted for by cell-associated antigens at any time; 3) within each cell line the ratios of extracellular to cell-associated antigen varied widely depending on the antigen considered.

Treatment with fibroblast IFN caused enhanced shedding of Ia, HLA-A,B,C antigens and β_2-m; treatment of M14 cells with leukocyte IFN caused only enhanced release of HLA-A,B,C and β_2-m, not of Ia antigens. Hence the pattern of enhancement of shedding paralleled that of increased expression at the cell level. This excludes the possibility that the larger amounts of antigens associated with the cells were simply due to inhibition of release, in keeping with very recent data on increased shedding of β_2-m after treatment with human leukocyte IFN [11].

Ia molecules, HLA-A,B,C antigens and β_2-m play an important role in the triggering and control of immune responses [1, 8, 21, 40, 52]. However, their specific functions, as far as they are known, have only been defined in qualitative terms. The quantitative aspects of their expression and shedding into the extracellular environment have not yet been investigated. Therefore, it is difficult to speculate as to what the significance of the IFN effects might be. Increased shedding of β_2-m was generally more pronounced than that of HLA-A,B,C molecules. One possible explanation might be an excess synthesis of free β_2-m chains, which would not associate with HLA-A,B,C heavy chains and would therefore be released in the medium at an increased rate after IFN treatment. Recently, it has been found that β_2-m, in addition to being associated with H-Y antigens, is also associated with TL-like molecules [30] in human cells and with Qa-2, TL and F9 molecules in mouse cells [9, 29, 42, 50]. It is possible, therefore, that increased β_2-m shedding reflects increased release of one or more of these molecules. A more detailed knowledge of the functions of membrane structures, especially those controlled by the MHC, is ob-

viously needed to judge the importance of these quantitative changes with respect to the mechanism of action of IFN-induced effects on the immune functions.

ACKNOWLEDGEMENTS

We are indebted to Dr. G.B. Rossi for reading this manuscript, and to Ms. Dora Centis for the excellent technical assistance.

This work was partially supported by a grant from CNR, Progetto Finalizzato Virus, Contratto No. 80.00386.84.

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